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Data in Brief

Differentially expressed microRNAs in colorectal cancer metastasis

Mohammed Abba [a,b], Axel Benner [c], Nitin Patil [a,b], Oliver Heil [d], Heike Allgayer [a,b,*]

[a] Department of Experimental Surgery, Medical Faculty Mannheim, University of Heidelberg, Germany
[b] Centre for Biomedicine and Medical Technology (CBTM), Mannheim, Germany
[c] Department of Biostatistics, German Cancer Research Centre (DKFZ), Heidelberg, Germany
[d] Genomics and Proteomics Core Facility, German Cancer Research Centre (DKFZ), Heidelberg, Germany

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ABSTRACT

Tumor metastasis continues to be the most significant contributor to cancer related mortality, and although several studies have examined expression profiles emanating from patients with metastatic disease, very little information is available about signatures that differentiate metastatic lesions from primary tumors and associated normal tissues, largely because such matched tissue sample series are rare. This study was specifically designed to identify the metastasis relevant microRNAs in colorectal cancer and characterize microRNAs that modulate the metastatic phenotype. Here we describe in detail how the data, deposited in the Gene Expression Omnibus (GEO) with the accession number GSE54088, was generated including the basic analysis as contained in the manuscript published in Cancer Research with the PMID 26069251.

Specifications	
Organism/cell line/tissue	Homo sapiens/colon and rectum
Sex	All male
Sequencer or array type	The Illumina human miRNA Sentrix Universal Array Matrix (SAM) V1 arrays
Data format	Raw and analyzed
Experimental factors	Tumor, normal, metastasis and background tissues
Experimental features	UICC stage IV
Consent	Informed consent and ethical approval obtained from patients and ethical committee, respectively
Sample source location	UMM Mannheim, Germany

1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE54088.

2. Experimental design, materials and methods

2.1. Experimental design

Tissue samples from colorectal cancer patients comprising the primary tumor, adjacent normal tissue, resected metastasis and the normal background tissue in which the metastasis occurred were obtained from and used for miRNA profiling (Fig. 1).

2.2. Materials and methods

2.2.1. Patient material

Tumor, metastasis, and corresponding normal and background tissue samples of patients with colorectal cancer were stored and obtained from the tumor bank of the Mannheim Medical Faculty, University of Heidelberg, Germany. The biobank was approved by the Ethical Committee of the Medical Faculty, University Hospital Mannheim, and informed consent was obtained from patients or their spouses when the former were deceased. Tissue sections and specimen were prepared by pathologists prior to snap freezing and subsequent storage in liquid nitrogen prior to sectioning. Biobanking and handling of the tissues followed the BRISQ guidelines [1]. 20–30, 20 μm sections/sample (depending on tissue size) were made in a cryotome and used for RNA extraction. All samples had at least 80% tumor cell content.

2.2.2. Patient characteristics

Nine patients in total were profiled, four with primary colonic tumors and six with rectal tumors. One patient had a primary tumor in both colon and rectum and accounts for the extrarectal tumor sample. All patients had stage IV disease with distant metastasis to either the liver or lung. 8 of these patients had additional mRNA profiling data and were included in the subsequent analysis as published in [2]. An overview of the patients is given in Table 1.

* Corresponding author at: Dept. of Experimental Surgery, Medical Faculty Mannheim, Ruprecht Karls University Heidelberg, Ludolf Krehl Str.13-17, 68167 Mannheim, Germany.
E-mail address: heike.allgayer@medma.uni-heidelberg.de (H. Allgayer).

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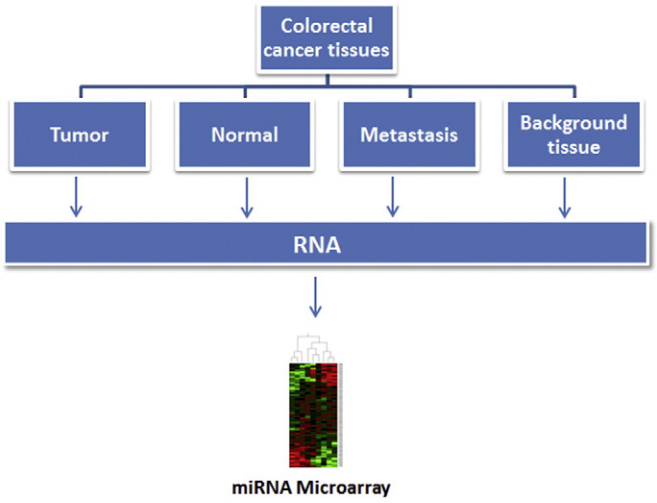

Fig. 1. Schematic representation of experimental flow. RNA was extracted from primary tumor, normal mucosa, and metastasis and background tissue of colorectal cancer patients. Total RNA was subsequently processed for miRNA and mRNA profiling using microarray platforms. The data presented in this manuscript is only for the miRNA data, in which 9 patients were profiled, but one was dropped for subsequent comparative analysis with the mRNA data.

2.2.3. RNA isolation

Total RNA, including small RNAs, was isolated using the miRNeasy mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Briefly, the thin cryosections were homogenized by vortexing in QIAzol lysis reagent followed by chloroform separation and column purification. RNA concentration was measured using the Nanodrop spectrophotometer, and RNA quality was evaluated on StdSens chips using lab-on-chip automated electrophoresis (BioRad, Germany). Only samples with an RNA quality index (RQI) greater than or equal to 7 were used for further downstream processing.

2.2.4. In vitro transcription, amplification, labeling and hybridization

In general, the Illumina human miRNA Sentrix Universal Array Matrix (SAM) protocol was used. Briefly, 200 ng total RNA was polyadenylated with a PAP enzyme (Poly-A Polymerase) and then reverse transcribed using biotinylated oligo-dT primers. The cDNA templates were then hybridized with miRNA-specific oligos (MSOs) and unbound oligos were washed away. After second-strand cDNA synthesis and PCR amplification with fluorescently labeled primers, the labeled products were hybridized and signals detected with the Illumina BeadScan reader. For quality assurance purposes, control samples were added and evaluated at the polyadenylation, first strand synthesis, hybridization of miRNA-specific oligos, second strand synthesis, fluorophore incorporation and array hybridization steps.

2.2.5. Data normalization

Quantile normalization was applied using all array data from the experiment. Resulting data were log2 transformed.

2.2.6. Statistical analysis

Pairwise comparisons were performed to test differential miRNA expression between paired samples. First, normal and tumor tissues were compared. We selected the colon samples from the patient where we had colon and rectum samples to get a design that was nearly balanced with respect to tumor site. Additional tests were performed for colon and rectum samples separately. Paired tests for differences between pairs of metastasis and corresponding normal tissue in the liver and lung were computed. Finally, we compared pairs of tumor samples and corresponding metastases. This was done as well for distant-site normal colorectal tissue samples corresponding to the primary tumor of the respective patients. We then identified those miRNAs with significant differences in the comparison of metastasis taking the differences between metastasis and the surrounding normal tissue into account. A multiplicity adjusted significance level of 5% was used in all comparisons. Venn diagrams were used to display comparatively the different sets of significant miRNAs. The statistical analyses were performed using the approach described by Smyth [3]. Adjustment for multiple testing was done by controlling the false discovery rate [4,5]. All statistical analyses were performed within the R statistical software environment (R version 2.15.3) using the R/Bioconductor packages beadarray [6], version 2.6.0, and limma, version 3.14.4. MicroRNAs with an estimated fold change of ≥1.5 and adjusted p-value ≤ 0.1 were considered for further evaluation.

3. Discussion

We have described here a very unique data set of patients with advanced colorectal cancer in which primary tumor and metastasis were evaluated alongside their corresponding normal samples. This data set is further supported by additional clinical data that could be exploited in deciphering clinical associations; however, the small sample size limits this applicability. This data set has been used as a source of hypothesis generation to discern and investigate microRNAs that impact metastasis in colorectal cancer. The outcome of such analyses has led to the generation of our recent manuscript [2].

Conflict of interest

The authors declare no conflict of interests.

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Table 1
Bio-sketch of profiled patients.

Sr. No	Anonymized IDs	Age*	Gender	Histology	Tumor presentation	Grade	TNM classification	UICC classification
1	P2	59	Male	Adenocarcinoma	Rectum	G 2	pT3, pN1, pM1	Stage IV
2	P4	76	Male	Adenocarcinoma	Sigmoid colon	G 2	pT3, pN1, pM1	Stage IV
3	P6	65	Male	Adenocarcinoma	Sigmoid colon	G 2	pT3, pN0, pM1	Stage IV
4	P7	65	Male	Adenocarcinoma	Rectum	G 2	pT3, pN0, pM1	Stage IV
5	P8	66	Male	Adenocarcinoma	Colon	G 2	pT4, pN1, pM1	Stage IV
6	P8	66	Male	Adenocarcinoma	Rectum	G 2	PT2, pN0, pM1	Stage IV
7	P9	71	Male	Adenocarcinoma	Sigmoid colon	G 3	pT4, pN2, pM1	Stage IV
8	P10	49	Male	Adenocarcinoma	Recto-sigmoid	G 2	pT3, pN1, pM1	Stage IV
9	P11	65	Male	Adenocarcinoma	Recto-sigmoid	G 2	pT3, pN2, pM1	Stage IV
10	P12	50	Male	Adenocarcinoma	Rectum	G 3	pT2, pN2, pM1	Stage IV

* Age at surgery in years.

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